

ORIGINAL RESEARCH ARTICLE

Quantifying the food requirements and effects of food stress on bumble bee colony development

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Electronic Supplementary Material

Table S1. Ad-lib worker number and colony weight ranges divided into five categories used for designating % Pollen (P) and BioGluc (G) quantities for each treatment. These quantities were based on feeding every 3 days.

Worker no.	5		6 - 14		15 - 25		30 - 40		40 +	
Weight (g)	Up to 24		24 - 35		35 - 56		56 - 69		69 - 112 +	
Treatment	P (g)	G (ml)	P (g)	G (ml)	P (g)	G (ml)	P (g)	G (ml)	P (g)	G (ml)
40%	0.63	2.64	2.10	6.86	2.29	11.14	6.00	19.54	7.99	16.46
52%	0.82	3.43	2.73	8.91	2.98	14.49	7.79	25.41	10.39	21.39
64%	1.01	4.22	3.35	10.97	3.66	17.83	9.59	31.27	12.79	26.33
76%	1.20	5.02	3.98	13.03	4.35	21.17	11.39	37.13	15.18	31.27
88%	1.39	5.81	4.61	15.09	5.03	24.51	13.19	42.99	17.58	36.21
100%	1.58	6.60	5.24	17.14	5.72	27.86	14.99	48.86	19.98	41.14

Table S2. PCR assays and cycling conditions for the 3 parasites analyzed in Experiments 1 and 2: *Nosema bombi*, *Nosema ceranae*, and *Crithidia bombi* (following Graystock et al. 2014).

PCR reactions (10 μ l)	<i>Nosema bombi</i>	<i>Nosema ceranae</i>	<i>Crithidia bombi</i>
dNTP/ MgCl ₂ (nM)	0.3/3.75	0.2/1.5	0.4/1.5
5xbuffer (μ l)	2	2	3
Taq (U)	0.25	2.5	1.25
Primer F/R (μ M)	0.2/0.2	0.2/0.2	0.5/0.5
Template (μ l)	2	1	2
PCR conditions			
Denaturing	95C, 4 s	95C, 5 s	94C, 2 s
Annealing	95/50/72C, 60 s	94/58/72C, 60 s	94/56/72C, 30 s
Extension	72C, 4 s	72C, 10 s	72C, 3 s

Table S3. Linear mixed-effect model for colony weight with pollen, nectar, time, time², no. workers (at start of experiment) as fixed effects (in Experiment 1), plus *N. ceranae* infection as fixed effects (in Experiment 2).

		Parameter Estimate ± SE	t-value	p-value
Experiment 1	(Intercept)	768.80 ± 132.64	5.796	<0.001
	Pollen %	-8.663 ± 1.796	-4.822	0.0001
	Nectar %	-7.446 ± 1.701	-4.378	0.0003
	Time	-52.27 ± 4.919	-10.62	<0.001
	Time ²	0.611 ± 0.144	4.239	<0.001
	Pollen*Time	0.392 ± 0.036	10.79	<0.001
	Nectar*Time	0.269 ± 0.032	8.312	<0.001
	Pollen*Nectar	0.077 ± 0.022	3.493	0.0022
Experiment 2	(Intercept)	514.86 ± 15.64	32.92	<0.001
	Pollen %	0.207 ± 0.124	1.676	0.104
	Nectar %	0.330 ± 0.125	2.636	0.013
	Time	-0.933 ± 0.744	-1.254	0.210
	Time ²	-0.062 ± 0.028	-2.177	0.030
	No. workers	0.923 ± 0.531	1.740	0.092
	<i>N. ceranae</i>	-11.45 ± 11.96	-0.958	0.346
	Pollen*Time	0.018 ± 0.005	3.866	0.0001
	Nectar*Time	0.079 ± 0.005	16.71	<0.001
	No. workers*Time	0.053 ± 0.020	2.635	0.009
	<i>N. ceranae</i> *Time	-1.726 ± 0.452	-3.819	0.0002

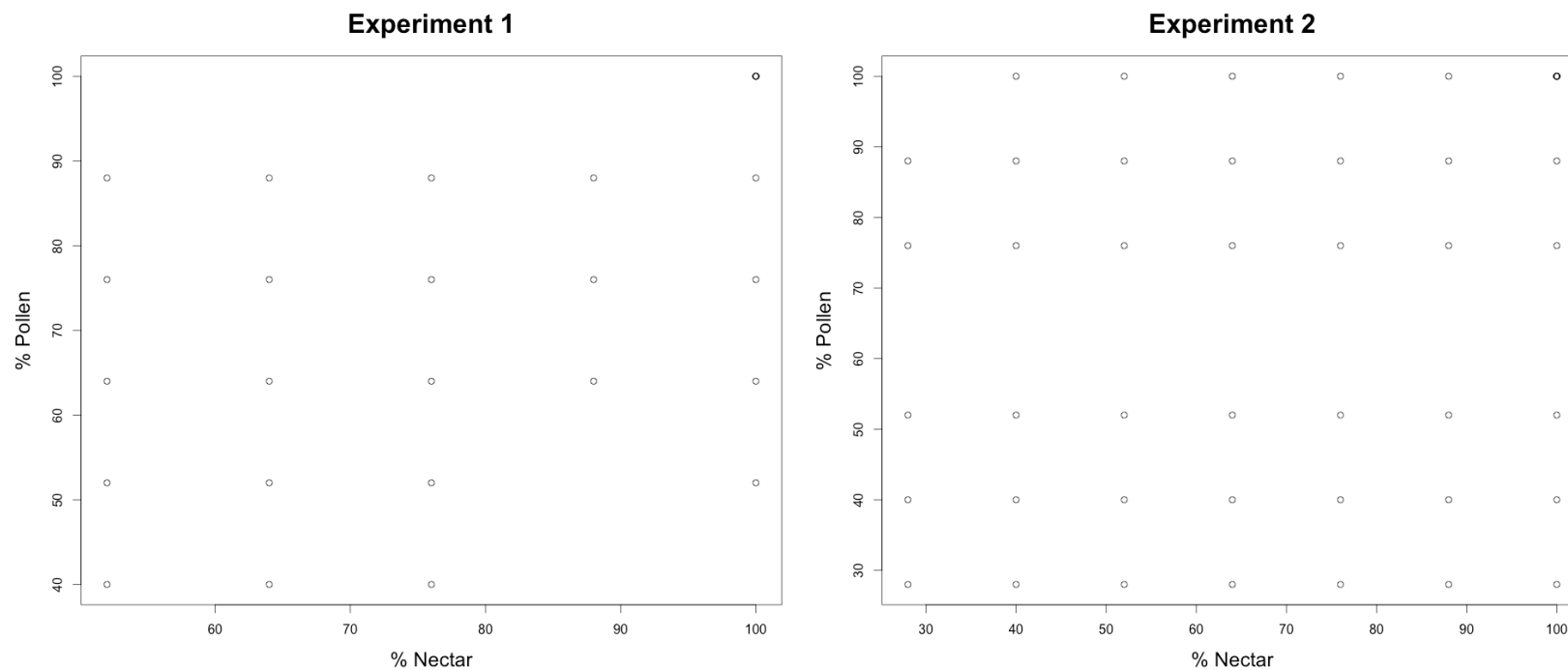


Figure S1. Scatterplot illustrating treatments in Experiments 1 (N=27 founded colonies from locally collected queens) and 2 (N=47 queen right founded factory colonies). NB. There were multiple ad-lib i.e. 100% nectar and pollen treatments (5 in Experiment 1, 7 in Experiment 2) and one colony assigned 28% Nectar 100% pollen did not grow so was removed from Experiment 2.

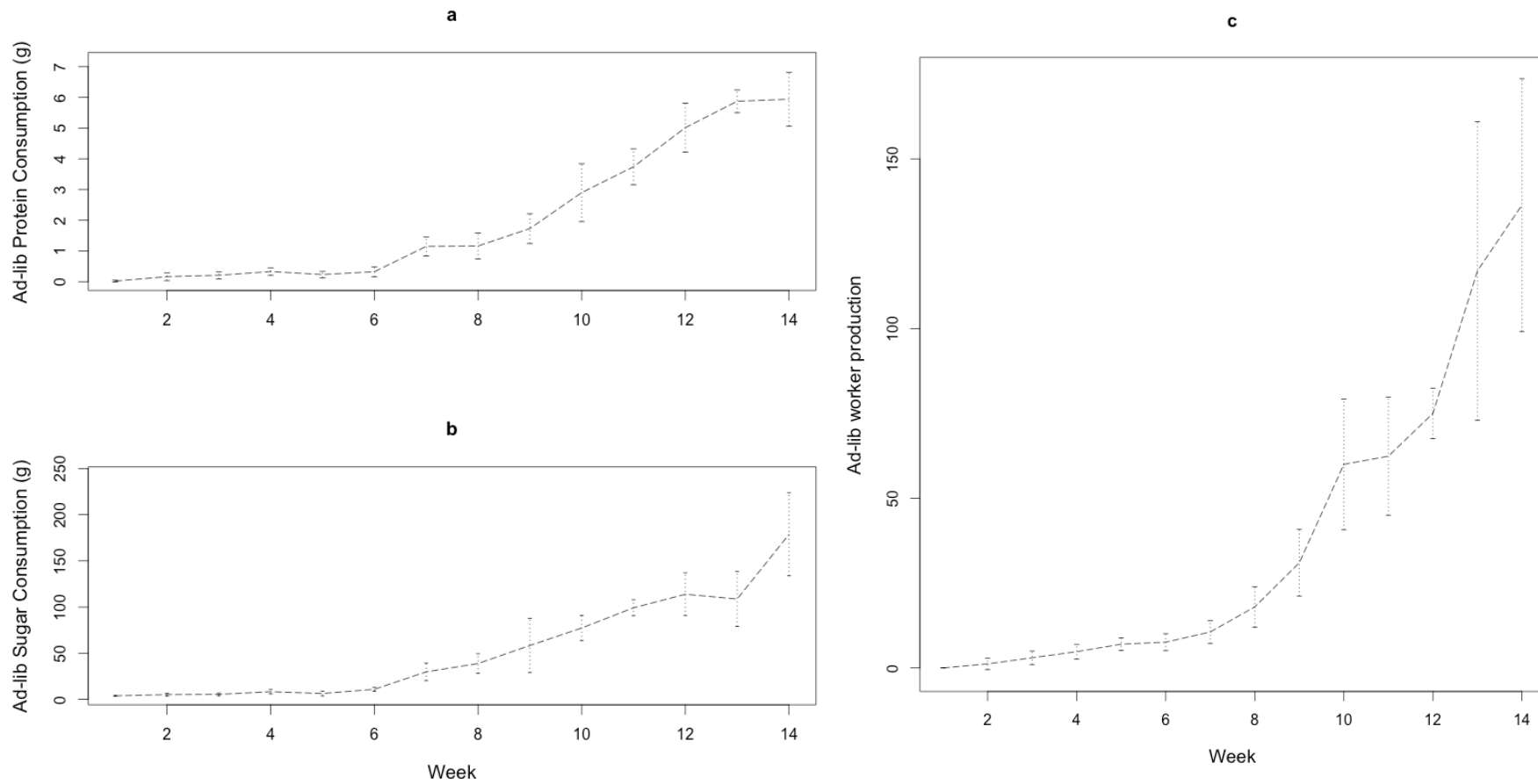


Figure S2. Line plots illustrating protein (a) and sugar (b) consumption, and worker production (c) per week (from founding 1.4.14 to expiration after 17.6.14) in ad-lib bumble bee colonies in Experiment I. NB. worker number was estimated from week 11 due to wax roofs obscuring the view of the nest, and male production – males weren't identified at this stage.

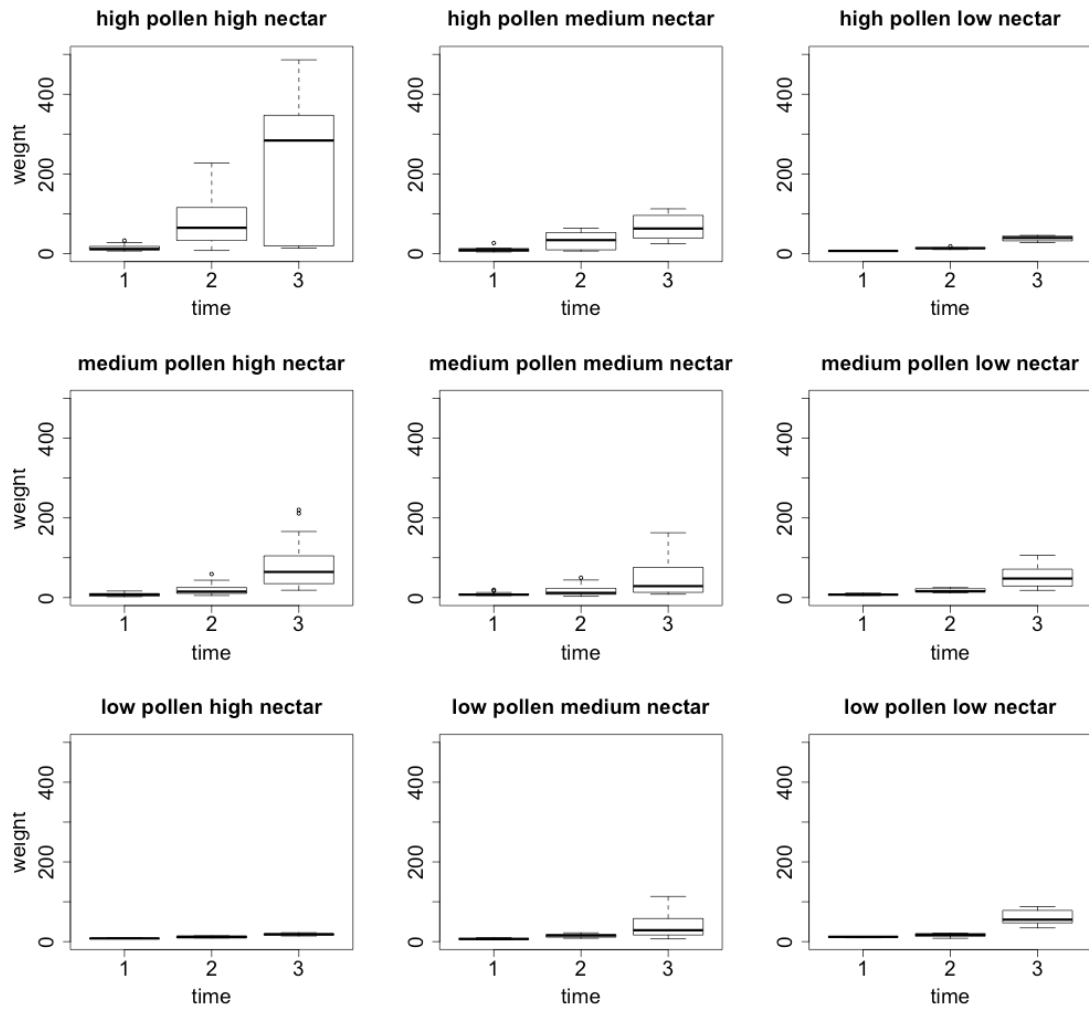


Figure S3. Boxplots to illustrate the effects of pollen and nectar on colony growth in Experiment I. To illustrate these effects, the data was divided into low (40 and 52), medium (64 and 76), and high (88 and 100) nectar and pollen treatment groups, and equal (six per group) consecutive time periods (1: 1 to 24 April, 2: 28 April to 3 June, 3: 10 June to 15 July).

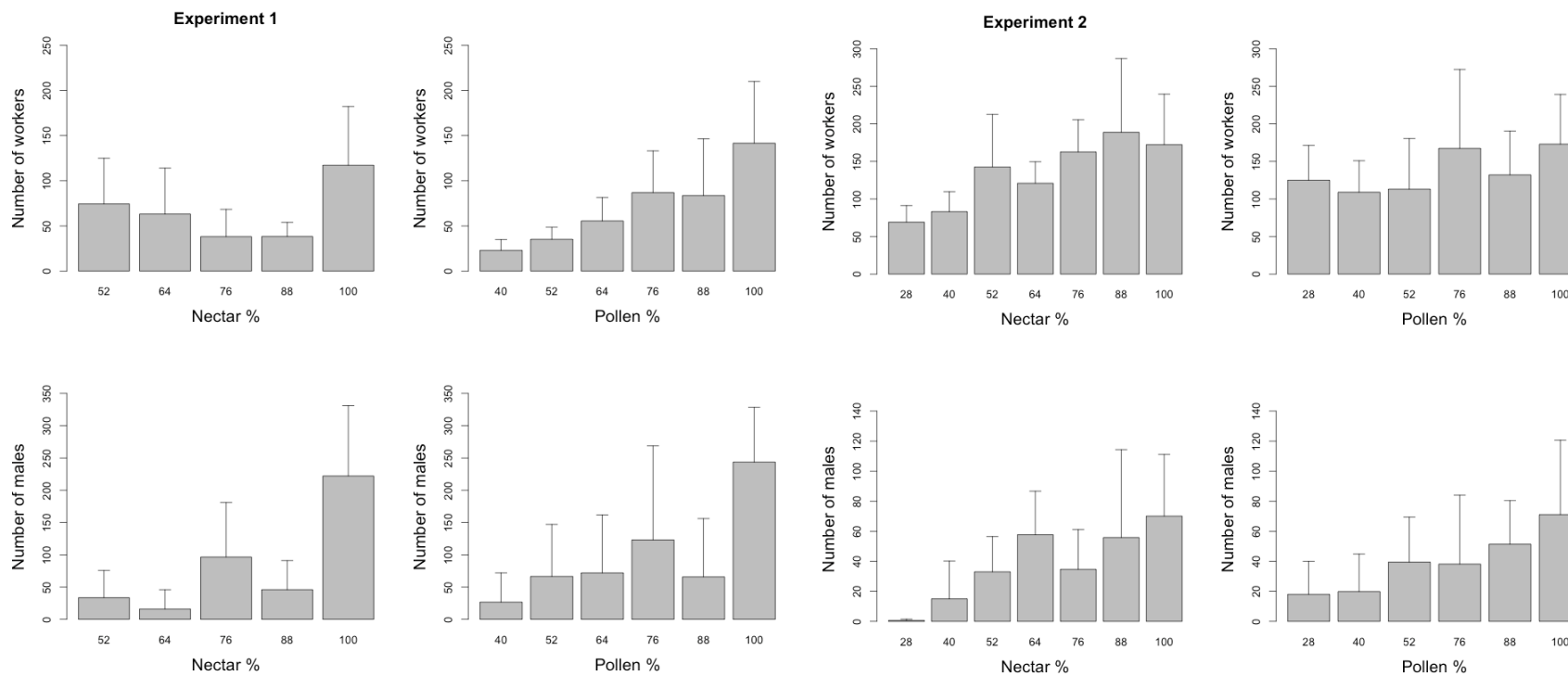


Figure S4. Barplots of mean worker and male production in different groups of bumble bee colony treatments with increasing proportions of pollen and nectar, plus standard deviation error bars, for Experiment 1 and 2. Note difference in scale of number of adults where more males were produced in Experiment 1, while more workers were produced in Experiment 2.